Prymnesium parvum population dynamics during bloom development: a role assessment of grazers and virus

Leslie Schwierzke[1], Daniel L. Roelke*[2], Bryan W. Brooks[3], James P. Grover[4], Theodore W. Valenti, Jr.[5], Mieke Lahousse[5], Carrie J. Miller[1], James L. Pinckney[6]

[1]Research Associate
Department of Wildlife and Fisheries Sciences
Texas A&M University, College Station, TX

[2]Associate Professor, *corresponding author
Departments of Wildlife and Fisheries Sciences and Oceanography
Texas A&M University
2258 TAMUS
College Station, TX 77843-2258, USA
Phone: (979) 845-0169; Fax: (979) 845-4096; e-mail: droelke@tamu.edu

[3]Associate Professor
Department of Environmental Science, and Center for Reservoir and Aquatic Systems Research
Baylor University, Waco, TX

[4]Professor
Department of Biology, and Program in Environmental and Earth Sciences
University of Texas at Arlington, Arlington, TX

[5]Research Associate
Department of Environmental Science, and Center for Reservoir and Aquatic Systems Research
Baylor University, Waco, TX

[6]Associate Professor
Marine Science Program and Department of Biological Sciences
University of South Carolina, Columbia, SC

Running head:
P. parvum field experiments

ABSTRACT

The toxic haptophyte *Prymnesium parvum* is a harmful alga known to cause fish-killing blooms that occur worldwide. In Texas (USA), *P. parvum* blooms occur in inland brackish water bodies and have increased in frequency and magnitude in recent years. In this study we conducted three consecutive field experiments (Lake Whitney) to investigate the influence of zooplankton and viruses on *P. parvum* bloom dynamics during the time of year when blooms are still typically active in Texas (early spring). A localized *P. parvum* bloom developed during our study that involved increasing levels of toxicity (based on *Pimephales promelas* and *Daphnia magna* bioassays). Only in our last experiment, during later stages of bloom development and under highly toxic conditions, did the presence of grazers show a statistically significant, negative effect on *P. parvum* population dynamics. During this experiment, a rotifer-dominated zooplankton community emerged, composed mostly of *Notholca laurentiae*, suggesting that this species was less sensitive than other grazers to toxins produced by *P. parvum*. Microzooplankton may have also been important at this time. Similarly, only our final experiment demonstrated a statistically significant, negative effect of viruses on *P. parvum*. This exploratory study, resulting in observed impacts on *P. parvum* populations by both grazers and virus, enhances our understanding of *P. parvum* ecology and highlights direction for future studies on resistance of zooplankton to prymnesin toxins and algal-virus interactions.

KEY TERMS: aquatic ecology; lakes; harmful algal blooms; toxicology; grazing; pathogens; phytoplankton; zooplankton; allelopathy; prymnesins

INTRODUCTION

Harmful algal blooms (HABs) have received the continued interest and concern of scientists, fisheries and water resource managers, and public health officials worldwide over the past two decades (Granéli and Turner 2006). Globally, aquatic ecosystems are being disrupted by the increasing incidence and severity of HABs (Smayda 1990; Hallegraeff 1993), which cause a variety of deleterious effects on aquatic systems, ranging from oxygen deficiency to human health risks (Van Dolah 2001; Granéli and Turner 2006). The haptophyte *Prymnesium parvum* is widely distributed, temperature tolerant (Baker et al. 2007), and capable of forming blooms in both coastal and brackish inland water bodies under varying salinity (Edvardsen and Paasche 1998; Lundholm and Moestrup 2006).

P. parvum blooms have been reported from estuarine and inland waters of the United States, Australia, China, Russia, Europe, Israel, Morocco, and other locations (Guo et al. 1996; Edvardsen and Paasche 1998). In the 1980s, the first noted blooms in brackish inland waters in the southwestern US occurred at numerous locations along the Pecos River (James and De La Cruz 1989). Since then, the state of Texas has experienced an increasing number of *P. parvum* blooms resulting in economic losses and massive fish kills. As of 2006 an estimated 31 million fish have been affected, with majority of the impact occurring in recent years (TPWD 2007). Confirmed blooms, where cell densities typically exceed 10×10^6 cells l^{-1}, can occur anytime throughout the year, but most of the blooms in Texas span the months between fall and spring, generally October through April (Joan Glass, TPWD, personal communication). Over 19 reservoirs along five major river systems have been affected in Texas.

Blooms of *P. parvum* persist under complex environmental conditions, and recent studies have revealed how this species might gain a selective advantage over other phytoplankton,

thereby accumulating biomass. The mixotrophic feeding strategies of *P. parvum* allow it to be photosynthetic and to feed phagotrophically, ingesting microorganisms such as bacteria and other protists (Nygaard and Tobiesen 1993; Tillmann 1998; Skovgaard and Hansen 2003). By producing allelopathic chemicals, *P. parvum* can immobilize plankton and suppress competitors, thereby fueling bloom development and persistence (Fistarol et al. 2003, 2005; Granéli and Johansson 2003; Uronen et al. 2005).

The toxicity of *P. parvum* blooms is caused by toxins that when released into the water can negatively impact some micro- and mesozooplankton. Field experiments performed by Roelke et al. (2007) revealed grazer inhibition and dramatically reduced grazer populations, with direct negative effects on cladoceran fecundity during a *P. parvum* bloom. Sopanen et al. (2006) found that exposure to *P. parvum* toxins caused inactivity and sublethal effects on the copepods *Eurytemora affinis* and *Acartia bifilosa*. Similar results were discovered in a study where the marine rotifer *Brachionus plicatilis* was negatively affected after ingesting toxic *P. parvum* cells (Barreiro et al. 2005). Furthermore, Brooks et al. (this issue) observed negative effects by *P. parvum* toxins on the freshwater rotifer *Brachionus calyciflorus*.

Factors controlling the termination of *P. parvum* blooms have not been well documented. However, various possibilities are suggested by several studies of *P. parvum* and related harmful algal species. Barkoh et al. (2003) treated samples from a fish hatchery with ammonium, reducing *P. parvum* populations to below detection with hemacytometer counts ($<10^5$ cells ml^{-1}) within 48 hours. More recently, ammonium doses added to laboratory batch cultures reduced toxicity and abundance of *P. parvum* (Grover et al. 2007). Similarly, field experiments indicated that nutrient additions reduced, and sometimes eliminated, *P. parvum*'s toxicity and competitive edge over other phytoplankton (Roelke et al. 2007; Errera et al. 2008). Mesocosm experiments

performed by Brussaard et al. (2005) tested the regulatory role of viruses on the prymnesiophyte *Phaeocystis globosa* finding that viral infection restricted bloom formation and eventually led to bloom termination.

This study reports findings from field experiments conducted in Lake Whitney (Texas, USA), one of the systems recently affected by *P. parvum* blooms. Our goal was to investigate the roles of grazers and viruses during the time of year when blooms are generally still active. This experimental approach was novel because natural plankton communities were manipulated under in-lake conditions, and the potential influence of both zooplankton and viruses on *P. parvum* dynamics was concurrently investigated. Response variables included phytoplankton biomass and assemblage composition, as well as zooplankton biovolume and community structure.

MATERIALS AND METHODS

Site Description

Lake Whitney is a reservoir on the Brazos River, Texas, USA, constructed in 1951. The lake receives drainage from an area of 42,107 km^2, has a capacity of 4.68×10^8 m^3, a surface area of 95 km^2, and a shoreline of 362 km (Bailes and Hudson 1982). We chose an area near the south shoreline of Walling Bend Island for our in-lake experiments (Figure 1). This location was selected because of the historically high *P. parvum* population densities typically observed there during the late-fall through early spring months (Joan Glass, TPWD, personal communication).

We performed three in-lake experiments during a 6-week period in spring 2007, each lasting 7 days. Significant phytoplankton and zooplankton responses were observed within a 7-d period during previous in-lake experiments from a nearby system (Roelke et al. 2007, Errera et al.

2008). Although, experimental artifacts were observed beyond a 7-d period in experiments employing smaller volume containers, therefore we selected a 7-d duration (Errera et al. 2008). Experiments were initiated February 22, and March 8 and 29. We used 24 transparent 25-l polycarbonate carboys during each study, each being filled to a final volume of 24.5 l with a small amount of air in the headspace for neutral buoyancy. Lake water was collected near our study site, away from the shoreline at a depth of 0.5 m, a location selected for the occurrence of *P. parvum* populations at the time of experiments (Joan Glass, TPWD, personal communication); surface waters were well-mixed at the time of experiments. The carboys were suspended in the near-surface water by tethering them to anchored floatation platforms that allowed free movement with wave turbulence, keeping the contents well-mixed. To simulate the natural light environment, carboys were covered with a neutral density screen, reducing surface light by ~55%. At this time of year, lake Secchi depths average ~1 m, giving a light extinction coefficient of ~1.7 m^{-1} (Wetzel 2001). Therefore, a 55% reduction in surface light would occur at ~0.5 m, the collection depth for experimental initiation lake waters.

Experimental Treatments

Each experiment comprised three main treatments, with each using four to six carboys. Treatments included: (1) the natural plankton assemblage containing ambient populations of *P. parvum* (unmanipulated lake water), (2) the natural plankton assemblage with reduction of large grazers by filtering whole lake water through a 20-µm mesh, and (3) reduction of pathogens by passing lake water through a tangential flow filtration system to remove viral-sized particles, with original phytoplankton and zooplankton assemblages restored. Our experimental design included a fourth treatment to investigate an interaction effect of reducing both large grazers and

pathogens, but due to methodological issues, this data was compromised and is not included in the analysis (see statistics description).

Creating the reduced pathogen treatments was a multi-step process, focused on reducing viruses. First, lake water was collected five days prior to each experiment and transported to the laboratory. Then tangential flow filtration, a technique used to manipulate viral particles in previous research (see Frazatti-Gallina 2004), was employed. Lake water was passed through a 0.65-µm membrane cassette followed by a 10-kDa cassette (Millipore, Pellicon 2). Virus counts were performed using fluorescence enumeration (described below) and confirmed this water to be virus free. At the initiation of each in-lake experiment, the tangential flow system was again used (0.65-µm cassette only) to separate the phytoplankton assemblage from the bulk of the natural virus assemblage. To accomplish this, we collected the retentate from the tangential flow system (phytoplankton-sized particles from 24.5 l of lake water concentrated to 1.0 l), which was then added to 23.5 l of virus free water, created previously in the laboratory. Thus, particles greater than 0.65 µm were restored to their original densities, with particles less than 0.65 µm being diluted to approximately 4% of their initial concentration. Therefore, we refer to these treatments as virus-reduced because the complete removal of viruses was not performed.

During tangential filtration, we discovered that the recovery efficiency of phytoplankton-sized particles in the retentate was inconsistent, and this resulted in variable initial phytoplankton population densities between carboys for these treatments. Tests of the potential deleterious impacts on phytoplankton concentration within the tangential flow system showed no significant influence. A treatment of recombined retentate and permeate from the tangential flow filtration was compared to the grazer-reduced treatment; over 7 days, no significant different was observed between treatments for either *P. parvum* population density or chlorophyll *a* ($p > 0.05$).

Response Variables

Response variables were sampled once from all carboys at the end of each 7-d experiment. Characterizations of plankton included estimates of biomass for total phytoplankton and higher taxonomic groups, enumerations of *P. parvum* population densities, and biovolumes of total zooplankton and taxonomic groups. Inorganic nutrients were measured, and ambient toxicity to fish and cladocerans was also determined. Initial conditions were characterized by measurements taken just prior to experimental initiation from source waters used in each treatment, and samples for these response variables were also collected from the lake at the termination of each experiment. A comparison of these in-lake samples with the control treatment at termination verified minimal bottle artifacts.

Estimates of total phytoplankton biomass and biomasses of aggregated taxa were determined from phytopigment concentration measurements following Pinckney et al. (1998), using CHEMTAX, a matrix factorization program that uses a steepest descent algorithm to determine the 'best fit' of an unknown sample to an initial estimate of pigment ratios for targeted algal taxa (Mackey et al. 1997; Wright et al. 1996). Cyanobacteria, euglenophytes, chlorophytes, prymnesiophytes, cryptophytes, diatoms and chrysophytes were analyzed because of their historical prevalence in Lake Whitney. See Roelke et al. (2007) for additional detail of the HPLC and CHEMTAX methods followed.

Water column chlorophyll *a* and phaeophytin concentrations were determined using standard fluorometric procedures. A 50 ml sample was filtered through 47 mm GF/F glass microfibre filters (n=3 per carboy) and frozen until analysis (within 48 h of collection). Pigments were extracted with 90% acetone, centrifuged, and analyzed using a fluorometer (APHA 1998). In experiments 2 and 3, chlorophyll *a* samples were collected at initiation from

each carboy to account for differences incurred through tangential flow manipulation and grazer reduction methods. For example, initial *P. parvum* cell density values were adjusted according to initial chlorophyll *a* data to account for the variable initial phytoplankton population densities in the virus-reduced carboys.

A 100 ml phytoplankton sample was collected from each well-mixed carboy and preserved using glutaraldehyde (5% v/v). Enumeration of *P. parvum* population density was performed using a settling technique (Utermöhl 1958). A 1.5 ml subsample was settled for a 24-h period, then counted using an inverted, phase-contrast light microscope (400x, Leica Microsystems). Depending of the density of material in the samples, between 5-25 randomly selected fields of view were counted, which resulted in ~200 *P. parvum* cells counted per sample.

Zooplankton samples were collected following two methods. For in-lake conditions, sampled at both initiation and termination of each experiment (six total data points), a Schindler trap (61-µm mesh size) was used to concentrate a 12 l lake sample (collected at 0.5 m depth) to 50 ml. At the termination of each experiment, a 10 l sample was removed from each carboy and filtered through the cod end portion of a Schindler trap (61 µm) and concentrated to 50 ml. Zooplankton samples were preserved in 2% buffered formalin (10% v/v). A subsample of 15 to 18 ml was settled for 24 h, then counted using an inverted, phase-contrast light microscope (40x and 200x, Leica Microsystems). For each individual counted, dimensions were measured corresponding to best-fit geometric shapes to estimate biovolume (Wetzel and Likens 1991). Zooplankton species were grouped into protozoa (mostly testate amoebae and tintinnids), total rotifers, total copepod adults, and copepod nauplii. Zooplankton densities during these experiments were low, and our enumeration technique resulted in ~20-50 individuals counted per sample.

To confirm the removal of viral-sized particles, a 2 ml sample was collected at the start of each experiment from waters passed through tangential flow filtration. All virus samples were flash frozen in liquid nitrogen and stored at -86°C. Slide preparation followed methods by Noble (2001) using the nucleic acid stain SYBR Gold (Molecular Probes, Inc.), and enumeration was performed using epifluorescent microscopy (1000x).

Samples for inorganic nutrients were filtered through GF/F glass microfibre filters (0.7-μm pore size), and the filtrate was frozen until analysis. Using autoanalyzer methodology (Armstrong & Sterns 1967; Harwood & Kuhn 1970), analyses included the sum of nitrate (NO_3-N) and nitrite (NO_2-N), ammonium (NH_4), and orthophosphate (PO_4).

Ambient toxicity to a cladoceran and a fish model was evaluated for initial conditions and from each experimental carboy. A standardized 24-h static acute-toxicity assay was used with the fathead minnow (*Pimephales promelas*) model (US EPA 2002), and a standardized 10-d static renewal chronic-toxicity test was used with a cladoceran (*Daphnia magna*) model (US EPA 1994) with minor modifications (Dzialowski et al. 2006). Samples were collected and transported to the laboratory where toxicity tests were initiated within 24 h. Ambient samples for both bioassays were diluted using a 0.5 dilution series with reconstituted hard water (RHW), prepared according to standardized procedures (US EPA 2002).

RHW was the control treatment for all toxicity assays. Alkalinity (mg l^{-1} as $CaCO_3$) and hardness (mg l^{-1} as $CaCO_3$) of RHW were measured potentiometrically and by colorimetric titration, respectively, before initiation of acute studies (APHA 1998). Specific conductance (μS cm^{-1}), pH, and dissolved oxygen (mg l^{-1}) of RHW were also measured before toxicity testing. Climate controlled chambers were utilized for all toxicity tests (25 ±1°C with a 16:8 hour light-dark cycle). Fathead minnow larvae less than 48 h old were fed newly hatched *Artemia nauplii*

two hours before initiation of testing (US EPA 2002). *D. magna* were fed a Cerophyll/green algal suspension daily, prepared according to methods reported previously (Hemming et al. 2002). LC_{50} values for fathead minnow toxicity tests were estimated as percentage of ambient sample using Probit (Finney 1971) or Trimmed Spearman Karber (Hamilton et al. 1977) methods. No Observed Adverse Effect Concentrations (NOAEC) and Lowest Observed Adverse Effect Levels (LOAEL) for cladoceran reproduction responses were determined using ANOVA with Dunnett's test (US EPA 2002)

Data Analysis

Relative changes in *P. parvum* densities and chlorophyll *a* concentrations were calculated as $(Y_{7d}-Y_{initial})/ Y_{initial}$, and differences between experimental treatments were tested for significance using an independent-samples t-test (SPSS). The original experimental plan comprised a 3 x 2 factorial design with the intention to analyze treatment effects with an ANOVA. However, during in-field initiation of the experiments, we discovered artifacts introduced by the tangential flow methodology, i.e., initial phytoplankton population densities were inconsistent in carboys involving the virus-reduced treatment. Initial conditions can strongly influence the outcome with this experimental design (Roelke et al. 2003; Roelke and Eldridge 2009), therefore we doubted the validity of a direct comparison between the effect of reduced grazers and reduced virus. Hence, we chose a more conservative statistical analysis with the t-test, where the effect of reduced grazers and reduced virus was only compared with the control.

RESULTS

In-situ lake conditions

A toxic *P. parvum* bloom formed during the period of our in-lake experiments, where typical *P. parvum* bloom characteristics were observed. An ongoing fish kill was recorded (composed of shad, *Dorosoma* sp.; buffalo, *Ictiobus* sp.; crappie, *Pomoxis* sp.; bullhead catfish, *Ameiurus* sp.; largemouth bass, *Micropterus salmoides*; and minnow, *Pimephales* sp.), and golden-colored waters with surface foam lines were observed. *P. parvum* population densities increased throughout the first few weeks of sampling, then leveled off at a maximum density of 37.8 x 10^6 cells l^{-1} at initiation of experiment 3. Chlorophyll *a* concentrations showed a similar trend, consistently increasing during our sampling dates. CHEMTAX data corresponded with this trend, showing that Prymnesiophytes dominated the assemblage at near monospecific levels of 96, 81, and 99% of the phytoplankton biomass during late February, and early and late March (Figure 2A). Cyanobacteria, Chlorophytes, and Chrysophytes composed the remaining small percentages of aggregated phytoplankton biomass.

Ambient toxicity bioassays confirmed the developing bloom with non-lethal conditions to *P. promelas* and moderate sublethal toxicity on *D. magna* reproduction observed at initiation of experiment 1, and then lake waters became more toxic to *D. magna* and *P. promelas* at initiation of experiment 2 and remained so through initiation of experiment 3 (Figure 3).

Concurrent with increasing *P. parvum* cell densities, the total zooplankton biovolume decreased during bloom formation. The total biovolume at the initiation of experiment 1 was 16.0 x 10^6 μm^3 l^{-1}, then a dramatic decrease from 23.1 x 10^6 to 1.86 x 10^6 μm^3 l^{-1} was observed between the last two experiments as waters became toxic (Figure 2B). A shift in the zooplankton composition also occurred. Rotifers dominated the community at the start of sampling, composed mostly of *Keratella* sp. (7.76 x 10^6 μm^3 l^{-1}, ~17 individuals l^{-1}) and *Notholca* sp. (5.50 x 10^6 μm^3 l^{-1}). Both *Notholca labis* (5.25 x 10^6 μm^3 l^{-1}, ~6 individuals l^{-1}) and *Notholca*

laurentiae (0.25 x 10^6 μm^3 l^{-1}, ~1 individuals l^{-1}) were present; this distinction between *Notholca* species was needed for interpretation of experimental results. At the onset of experiment 2, the zooplankton community was dominated by copepod nauplii (11.7 x 10^6 μm^3 l^{-1}, ~15 individuals l^{-1}). Adult copepods and rotifers were subdominant, with *Keratella* sp. and *N. laurentiae* representing the rotifer composition equally. At the initiation of experiment 3, copepod nauplii were still dominant (1.33 x 10^6 μm^3 l^{-1}, ~2 individuals l^{-1}), but the subdominant rotifer composition exhibited *N. laurentiae* dominance (0.38 x 10^6 μm^3 l^{-1}), with *Keratella* sp. present as well (0.09 x 10^6 μm^3 l^{-1}).

The physicochemical environment may have subtly influenced the bloom formation. Ammonium ranged between 1.09 and 1.28 μmol l^{-1}, while nitrate and nitrite ranged 0.06 to 0.13 μmol l^{-1}. Phosphate increased from 0.05 to 0.10 μmol l^{-1} during sampling (Figure 4A). Nutrients were not likely growth limiting to *P. parvum* based on half-saturation coefficients for the Texas strain, i.e., $K_N = 0.005$ and $K_P = 0.0068$ (Baker 2007; Baker et al. *In press*). Throughout the experiments Secchi depth was relatively deep for this region (averaging ~1 m), where lakes are generally turbid, suggesting that light was also not a limiting factor (Figure 4B). Temperatures steadily increased from 9 to 17ºC, and pH values remained between 8.1 and 8.6, peaking during experiment 2 (Figure 4C).

Experimental Results

The first treatment comparison focuses on the role of grazers, evaluating differences between reduced grazers and the control. Experiment 1 was initiated during less toxic conditions, and *P. parvum* population density increased in both the natural assemblage (NA) and the reduced grazer (RG) treatments; no significant difference was observed between these treatments (Figure 5A). Increases in population density ranged from 15-50%, to an average of

~32 x 10^6 cells l^{-1}. Chlorophyll *a* concentration decreased in both NA and RG treatments, and no significant difference between treatments was observed (Figure 5A). Decreases in concentration ranged from 5-20%, to an average of ~29 μg l^{-1}. In the NA treatment, total zooplankton biovolume increased between 50-130%, to an average of ~32 x 10^6 μm^3 l^{-1}, and no compositional shift was observed. Rotifers dominated the community, averaging ~31 x 10^6 μm^3 l^{-1}, and were composed mostly of *Keratella* sp., *Notholca* sp., and *Brachionus* sp. (Figure 6A). In the RG treatment, zooplankton composition showed similar rotifer dominance at the conclusion of the experiment (Figure 5A). Interestingly, by the end of the first experiment, toxicity to both *D. magna* and *P. promelas* was observed, but no differences in toxicological benchmark concentrations (e.g., LC_{50}, NOAEL values) were observed between the NA and RG treatments.

In experiment 2, initial lake waters were toxic to both *D. magna* and *P. promelas* with a fish LC_{50} value of 1.9 % of ambient sample. *P. parvum* population density increased in both NA and RG treatments, and no significant difference between these treatments was observed (Figure 5B). Increases in population density ranged from 2-45%, to an average of ~43 x 10^6 cells l^{-1}. Chlorophyll *a* concentration decreased in both NA and RG treatments, and again, the difference between treatments was not significant (Figure 5B). Concentrations decreased between 2-10%, to an average of ~34 μg l^{-1}. An overall biovolume decline of 50-75% was observed in the NA treatment for total zooplankton. Copepod nauplii declined 88-94%, but rotifers, composed mostly of *Keratella* sp. and *N. laurentiae*, remained relatively unchanged during experiment 2 (Figure 6B). Again, similar zooplankton composition was observed in the RG treatment (Figure 5B). Consistent with ambient toxicity observed in experiment 1, experimental units were toxic to *P. promelas* and *D. magna* at the termination of this study, with LC_{50} values less than 1.5 % of

ambient sample, but again there were no differences in LC_{50} values between the NA and RG treatments.

The *P. parvum* bloom continued to develop, and the lake water remained toxic to *D. magna* and *P. promelas* with a fish LC_{50} value of 3.86 % of ambient sample at the initiation of experiment 3. A significant difference between NA and RG treatments was observed for *P. parvum* population density (Figure 5C), where greater *P. parvum* densities were measured in the RG treatment ($p = 0.038$). With grazers reduced, increases in population density ranged from 10-25%, to an average of ~45 x 10^6 cells l^{-1}, while *P. parvum* population density in the NA treatment ranged from a decrease of 10% to an increase of 15% (an average of ~39 x 10^6 cells l^{-1}). Chlorophyll *a* concentration decreased in both NA and RG treatments, and the difference between treatments was not significant (Figure 5C). Overall, reductions in chlorophyll *a* ranged from 2-14%, to an average of ~38 µg l^{-1}. During experiment 3, total zooplankton biovolume increased 3-to 4-fold in the NA treatment. This was due in large part to the accumulation of *N. laurentiae*, which averaged 5.8 x 10^6 μm^3 l^{-1}, or ~6 individuals l^{-1} (Figure 6C); copepod nauplii biovolume declined 87-100%. In the RG treatment, zooplankton composition showed similar rotifer dominance at the conclusion of the experiment (Figure 5C). Similar to experiments 1 and 2, high ambient toxicity to *P. promelas* and *D. magna* was observed in the experimental units at the end of study 3, with LC_{50} values less than 1.3 % of ambient sample and no differences in LC_{50} values between the NA and RG treatments.

Experiment 3 differed from the other experiments in that a large cell-sized component of the phytoplankton (~11% of the total chlorophyll *a*) was removed from the lake water during the grazer reduction step. This is indicated by a difference in initial chlorophyll *a* levels between NA and RG treatments.

Our second comparison of complementary treatments focused on the role of viruses, highlighting the differences between natural assemblage (NA) and virus-reduced (VR) treatments. Due to methodological artifacts, only data from the final two experiments are shown. During experiment 2, *P. parvum* population densities and chlorophyll *a* concentrations changed little in the NA and VR treatments, and no significant difference between these treatments was observed. Rotifers dominated the zooplankton community in both treatments at the end of this experiment (Figure 7A). All carboy contents were toxic to *P. promelas* and *D. magna*, but no differences were observed between NA and VR treatments.

In experiment 3, a significant difference between NA and VR treatments was observed for *P. parvum* population density (Figure 7B), where greater changes in *P. parvum* population densities were measured in the VR treatment ($p = 0.02$). Under reduced virus conditions, increases in population density ranged from 35-65%, to an average of ~39 x 10^6 cells l^{-1}, while *P. parvum* population density in the NA treatment ranged from a decrease of 10% to an increase of 15%. Chlorophyll *a* concentration decreased in both NA and VR treatments, and the difference between treatments was not significant. The zooplankton composition was very similar in both of these treatments, showing rotifer-dominant communities (Figure 7B). Again, all contents were toxic to *P. promelas* and *D. magna* at the end of this study.

DISCUSSION

During the period of our experiments, fish-killing *P. parvum* blooms occurred intermittently throughout Lake Whitney, with a developing bloom recorded at our study site. Zooplankton populations dramatically declined with the onset of in-lake toxicity. Interestingly, grazers significantly impacted *P. parvum* in the third experiment, where the reduction of grazers resulted

in an increase of *P. parvum* population density. During our final experiment, the observed compositional shift to a rotifer-dominated zooplankton community, almost exclusively *N. laurentiae* (or possibly a smaller micrograzer, less than 61 μm), alongside the near complete decline of copepod nauplii, suggests that *N. laurentiae* and perhaps smaller grazers were less sensitive to toxins produced by *P. parvum* than other grazers. Conversely, a recent toxicity study by Brooks et al. (this issue) documented a negative effect on population growth of the freshwater rotifer *Brachionus calyciflorus* when exposed to *P. parvum* toxins from the TX strain. Our findings imply that rotifer sensitivities to *P. parvum* toxins may be species-specific, with *N. laurentiae* showing an ability to develop tolerance and potentially resistance to prymnesin toxins and demonstrate population growth under ambient conditions that are toxic to fish and other zooplankton.

How might these observed zooplankton community shifts compare with seasonal changes that occur dependently or independently of *P. parvum* blooms? For this study, no previous data were available for *N. laurentiae* abundances during bloom and non-bloom events. However, our on-going research currently performs monthly whole system water quality monitoring in Lake Whitney, with fixed-station sites where zooplankton communities are sampled throughout the year (Roelke, unpublished data). With this larger data set, we will better understand the seasonal shifts in zooplankton community structure, specifically of *N. laurentiae*.

While our reduced-grazer treatments improved *P. parvum* performance, this outcome did not carry over to the entire phytoplankton community. CHEMTAX estimates suggested that Prymnesiophytes dominated throughout the experiments, but we observed decreases of chlorophyll *a* in the grazer-reduced treatment concurrent with increases in *P. parvum* population densities. This could indicate that *P. parvum* had a negative effect on other algae, possibly

through competition or allelopathy (Fistarol et al. 2003, 2005; Granéli and Johansson 2003; Roelke et al. 2007; Errera et al. 2008).

The effect of viruses on phytoplankton dynamics is a developing field of study, but research has shown that viruses can negatively impact phytoplankton populations. Brussaard et al. (2005) found that viral infection can restrict the growth of the prymnesiophyte *P. globosa*, another HAB-causing species closely related to *P. parvum*, but there is no previous evidence for viral effects on *P. parvum* dynamics. Our experiments demonstrated that viral manipulation appears to affect *P. parvum* populations. During the last experiment, carboys with a reduction in virus populations resulted in an improved *P. parvum* performance. Although not a dramatic increase in population growth, this observation still suggests a negative impact of viruses on *P. parvum*.

The effect of both grazers and viruses on *P. parvum* was more pronounced, and statistically significant, in the third experiment, relative to the second, even though both experiments exhibited near identical lethal and sublethal toxicity to *P. promelas* and *D. magna*, respectively, indicating that extracellular toxin levels were likely similar during experiments 2 and 3. In these final two experiments, the total zooplankton biovolume increased only in experiment 3, most likely indicating more grazing pressure on phytoplankton communities. Under these ambient toxicity conditions, it is possible that the compositional shift among rotifers toward *N. laurentiae* dominance (or perhaps smaller micrograzers) resulted in a stronger and more significant effect of grazer reduction on *P. parvum* demographics. This potential reduced sensitivity of freshwater *N. laurentiae* to *P. parvum* toxins and the role of microzooplankton both merit further investigation.

In the case of viruses, the observed increase in *P. parvum* population densities during later stages of bloom development (under reduced viral conditions) could indicate direct interactions between viruses and *P. parvum*; conversely, the reduction of viruses may have increased

bacterial densities (Schwalbach et al. 2004), therefore causing an indirect positive facilitation of *P. parvum*, known to utilize phagotrophic feeding strategies to prey on bacteria (Nygaard and Tobiesen 1993; Skovgaard and Hansen 2003). A review by Salomon and Imai (2006) highlights that phytoplankton viruses, an important factor in bloom termination, are likely to negatively impact blooms during later stages. Over the span of our experiments, it may be that increased viral infection and cell lysis began to affect *P. parvum* populations by the third experiment, and perhaps both grazer and virus impacts on *P. parvum* would increase in magnitude as the bloom continued to mature. Further research is essential to better understand the interactions of algal virus ecology involving *P. parvum* bloom dynamics.

Based on observations in Lake Whitney, the timing of onset and termination of *P. parvum* blooms varies locally. During the course of these experiments a localized bloom formed at our study site, while in other areas of the lake blooms formed earlier and were more persistent (Bryan Brooks, unpublished data; Joan Glass, TPWD, personal communication). Concentrations of dissolved nitrogen and phosphorus during the study period remained above those limiting the growth rate of *P. parvum* in laboratory cultures (Baker 2007; Baker et al. *In press*), which produced toxic cells under nutrient replete conditions when the salinity, light, and temperature environment became stressful (Baker et al. 2007). Although temperatures during these experiments were suboptimal, based on laboratory cultures, they would support specific growth at approximately half the optimal rate of about 0.9 d^{-1} (Baker et al. 2007). Using equations that estimate the average underwater irradiance (approximated with surface irradiance and a relationship between the light extinction coefficient and Secchi depth), the average irradiance in the lake was 183 uE m^{-2} s^{-1} (Kirk 1994; Wetzel 2001). By comparison to growth in laboratory cultures, the in-lake irradiance was above the light conditions (140 uE m^{-2} s^{-1}) used in our routine

culturing of *P. parvum* (Baker et al. 2007; Roelke et al. 2007); therefore not likely restricting the growth of *P. parvum*. Thus, it does not appear that one single factor triggered bloom development, in regards to temperature, light, pH, and nutrients, but maybe subtle changes in each of these parameters, as well as variable(s) not measured here, played a role in bloom dynamics.

P. parvum populations had already reached typical bloom densities at the initiation of experiment 1, yet bioassays showed non-toxic conditions to *P. promelas* and moderate effects on *D. magna* reproduction. Two weeks later, at experiment 2 initiation, lake waters were toxic to both *D. magna* and *P. promelas*. This localized development was likely triggered by a multitude of abiotic and biotic parameters, and discerning a specific factor that influenced bloom formation is difficult with the available data. If we assume the same chemicals resulting in toxicity also have an allelopathic effect (Fistarol et al. 2003, 2005; Granéli and Johansson 2003; Roelke et al. 2007; Errera et al. 2008), the lack of toxicity during the first experiment suggests that allelopathy played a minimal role in bloom development; however, these specifics involving *P. parvum* allelopathy require greater understanding. Possibly, the cooler in-lake temperatures hindered grazers preferring *P. parvum* as prey, thus enabling *P. parvum* population densities to reach bloom proportions.

The environmental conditions surrounding *P. parvum* bloom formation and termination seem complex. Our results from this exploratory research demonstrate grazer and virus impacts on *P. parvum* dynamics during toxic bloom conditions, and although follow-on research is necessary, these findings have potential implications for alga management strategies. The rise of *N. laurentiae*, apparently less sensitive to *P. parvum* toxins than other grazers, could have brought an effective grazer into the zooplankton community; this factor, in conjunction with the

proliferation of viruses affecting *P. parvum*, could possibly curb the magnitude of a bloom. Future research initiatives could focus on continued grazer and virus affects under maturing bloom conditions, and perhaps a more detailed look at interactions between phytoplankton and virus, and their affect on *P. parvum* populations.

ACKNOWLEDGEMENTS

We are grateful to Hsiu-Ping Li for her assistance with processing samples and to the personnel at the Cliffview Resort on Lake Whitney for their hospitality. The Texas Parks and Wildlife Department supported this research, with additional support from the U.S. Environmental Protection Agency and a federal initiative through the Department of Energy.

REFERENCES

APHA, 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.

Armstrong, F.A., C.R. Sterns, 1967. The measurement of upwelling and subsequent biological processes by means of the Technicon Autoanalyzer and associated equipment. Deep-Sea Res. I 14: 381-389.

Bailes, C., and D.L. Hudson, 1982. A Guide to Texas Lakes, including the Brazos, Colorado, Frio, and Guadalupe Rivers. Houston: Pacesetter Press.

Baker, J.W., 2007. Basic Ecology and Mathematical Modeling of *Prymnesium parvum*, "Golden Algae", in Texas. Ph.D. Dissertation, The University of Texas at Arlington, Arlington, Texas, USA. Chap. 4

Baker, J.W., J. P. Grover, B. W. Brooks, F. Ureña-Boeck, D. L. Roelke, R. M. Errera, R. Kiesling, 2007. Growth and toxicity of *Prymnesium parvum* (Haptophyta) as a function of salinity, light and temperature. J. Phycol. 43: 219-227.

Baker J.W., J.P. Grover, R. Ramachandrannair, C. Black, T.W. Valenti, Jr., B.W. Brooks, D.L. Roelke, *In press*. Dynamics at the edge of the niche: an experimental study of the harmful alga *Prymnesium parvum*. Limnol. Oceanogr.

Barkoh, A., D.G. Smith, J.W. Schlechte, 2003. An effective minimum concentration of un-ionized ammonia nitrogen for controlling *Prymnesium parvum*. N. Am. J. Aquacul. 65: 220-225.

Barreiro, A., C. Guisande, I. Maneiro, T.P. Lien, and 5 others, 2005. Relative importance of the different negative effects of the toxic haptophyte *Prymnesium parvum* on *Rhodomonas salina* and *Brachionus plicatilis*. Aquat. Microb. Ecol. 38: 259-267.

Brooks, B.W., S. James, T.W. Valenti Jr., F. Urena-Boeck, C. Serrano, L. Schwierzke, L.D. Mydlarz, J.P. Grover, D.L. Roelke, this issue. Comparative Toxicity of *Prymnesium parvum* in Inland Waters. Journal of American Water Resources Association.

Brussaard, C.P.D., B. Kuipers, M.J.W. Veldhuis, 2005. A mesocosm study of *Phaeocystis globosa* population dynamics I. Regulatory role of viruses in bloom control. Harmful Algae 4: 859-874.

Dzialowski, E.M., P.K. Turner, B.W. Brooks, 2006. Physiological and reproductive effects of β-adrenergic receptor antagonists on *Daphnia magna*. Arch. Environ. Contam. Toxicol. 50:503-510.

Edvardsen, B. and E. Paasche, 1998. Bloom dynamics and physiology of *Prymnesium* and

Chrysochromulina. In: *The physiological ecology of harmful algal blooms*, D.M. Anderson, A.D. Cembella, G.M. Hallegraeff (Editors). Springer, Berlin, Germany, pp. 193-208.

Errera, R.M., D.L. Roelke, R. Kiesling, B.W. Brooks, J.P. Grover, L. Schwierzke, F. Urena-Boeck, J.W. Baker, J.L. Pinckney, 2008. The effect of imbalanced nutrients and immigration on *Prymnesium parvum* community dominance and toxicity: results from in-lake microcosm experiments. Aquat. Microb. Ecol. 52: 33-44.

Finney, D. J, 1971. Probit analysis, 3rd ed. Cambridge University Press, London.

Fistarol, G.O., C. Legrand, E. Granéli, 2003. Allelopathic effect of *Prymnesium parvum* on a natural plankton community. Mar. Ecol. Prog. Ser. 255: 115-125.

Fistarol, G.O., C. Legrand, E. Granéli, 2005. Allelopathic effect on a nutrient-limited phytoplankton species. Aquat. Microb. Ecol. 41: 153-161.

Frazatti-Gallina, N.M., R.M. Mourao-Fuches, R.L. Paoli, 2004. Vero-cell rabies vaccine produced using serum-free medium. Vaccine 23: 511-517.

Granéli, E., N. Johansson, 2003. Increase in the production of allelopathic substances by *Prymnesium parvum* cells grown under N- and P-deficient conditions. Harmful Algae 2: 135-145.

Granéli, E. and J.T. Turner, 2006. Ecology of Harmful Algae. Springer, Berlin, Germany.

Grover, J.P., J. Baker, F. Ureña-Boeck, B.W. Brooks, R.M. Errera, D.L. Roelke, R. Kiesling, 2007. Laboratory tests of ammonium and barley straw extract as agents to suppress abundance of the harmful alga *Prymnesium parvum* and its toxicity to fish. Water. Res. 41: 2503-2512.

Guo, M., P.J. Harrison, F.J.R. Taylor, 1996. Fish kills related to *Prymnesium parvum* N. Carter (Haptophyta) in the Peoples Republic of China. J. Appl. Phycol. 8: 111-117.

1 Hallegraeff, G. M, 1993. A review of harmful algal blooms and their apparent global increase.
2 Phycologia 32: 79-99.
3 Hamilton, M.A., R.C. Russo, R.V. Thurston, 1977. Trimmed Spearman-Karber method for
4 estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol.
5 117: 714-719; correction 12: 417, 1978.
6 Harwood, J.E., A.L. Kuhn, 1970. A colorimetric method for ammonia in natural waters. Water.
7 Res. 4: 805-811.
8 Hemming, J.M., P.K. Turner, B.W. Brooks, W.T. Waller, T.W. La Point, 2002. Assessment of
9 toxicity reduction in wastewater effluent flowing through a constructed wetland using
10 *Pimephales promelas*, *Ceriodaphnia dubia*, and *Vibrio fischeri*. Arch. Environ. Contam.
11 Toxicol. 42: 9-16.
12 James, T.L., A. De La Cruz, 1989. *Prymnesium parvum* Carter (Chrysophyceae) as a suspect of
13 mass mortalities of fish and shellfish communities in western Texas. Texas. J. Sci. 41: 429-
14 430.
15 Kirk, J.T.O., 1994. Light and Photosynthesis in Aquatic Ecosystems, 2nd edition. Cambridge
16 University Press, Cambridge, UK.
17 Lundholm, N. and O. Moestrup, 2006. The Biogeography of Harmful Algae. In: *Ecology of*
18 *Harmful Algae*, E. Granéli and J.T. Turner (Editors). Springer, Berlin, Germany, pp. 23-35.
19 Mackey, M., D. Mackey, H. Higgins, S. Wright, 1997. CHEMTAX- a program for estimating
20 class abundances from chemical markers: application to HPLC measurements of
21 phytoplankton. Mar. Ecol. Prog. Ser. 144: 265-283.
22 Noble, R.T, 2001. Enumeration of Viruses. In: *Methods in Microbiology*, J.H. Paul (Editor).
23 Academic Press, pp. 43-51.

Nygaard, K. and A. Tobiesen, 1993. Bacterivory in algae- a survival strategy during nutrient limitation. Limnol. Oceanogr. 33: 823-847.

Pinckney, J., D. Millie, K. Howe, H. Paerl, J. Hurley, 1996. Flow scintillation counting of ^{14}C-labeled microalgal photosynthetic pigments. J. Plankton Res. 18: 1867-1880.

Pinckney, J., H. Paerl, M.B. Harrington, K. Howe, 1998. Annual cycles of phytoplankton community structure and bloom dynamics in the Neuse River Estuary, North Carolina. Mar. Biol. 131: 371-382.

Roelke, D.L., S. Augustine, Y. Buyukates, 2003. Fundamental predictability in multispecies competition: The influence of large disturbance. Am. Nat. 162: 615-623.

Roelke, D.L., R.M. Errera, R. Kiesling, B.W. Brooks, J.P. Grover, L. Schwierzke, F. Ureña-Boeck, J. Baker, J.L. Pinckney, 2007. Effects of nutrient enrichment on *Prymnesium parvum* population dynamics and toxicity: results from field experiments, Lake Possum Kingdom, USA. Aquat. Microb. Ecol. 46: 125-140.

Roelke, D.L., P.M. Eldridge, 2009. Losers in the 'Rock-Paper-Scissors' game: The role of non-hierarchical competition and chaos as biodiversity sustaining agents in aquatic systems. Ecol. Mod. *In Press*.

Salomon, P.S. and I. Imai, 2006. Pathogens of harmful microalgae. In: *Ecology of Harmful Algae*, E. Granéli and J.T. Turner (Editors). Springer, Berlin, Germany, pp. 271-282.

Schwalbach, M.S., I. Hewson, J.A. Fuhrman, 2004. Viral effects on bacterial community composition in marine plankton microcosms. Aquat. Microb. Ecol. 34: 117-127.

Skovgaard, A. and P.J. Hansen, 2003. Food uptake in the harmful alga *Prymnesium parvum* mediated by excreted toxins. Limnol. Oceanogr. 48: 1161-1166.

Smayda, T.J, 1990. Novel and nuisance phytoplankton blooms in the sea: evidence for a global

epidemic. In: *Toxic Phytoplankton Blooms*, E. Granéli, B. Sundström, L. Edler, D.M. Anderson (Editors). Elsevier, New York, USA, pp. 29-40.

Sopanen, S., M. Koski, P. Kuuppo, P. Uronen, C. Legrand, T. Tamminen, 2006. Toxic haptophyte *Prymnesium parvum* affects grazing, survival, egestion and egg production of the calanoid copepods *Eurytemora affinis* and *Acartia bifilosa.* Mar. Ecol. Prog. Ser. 327: 223-232.

Tillmann, U, 1998. Phagotrophy by a plastidic haptophyte, *Prymnesium patelliferum*. Aquat. Microb. Ecol. 14: 155-160.

TPWD (Texas Parks and Wildlife Department), 2007. Texas Harmful Algal Bloom Workgroup. Technical report #GI-378.

Uronen, P., S. Lehtinen, C. Legrand, P. Kuuppo, T. Tamminen, 2005. Haemolytic activity and allelopathy of the haptophyte *Prymnesium parvum* in nutrient-limited and balanced growth conditions. Mar. Ecol. Prog. Ser. 299: 137-148.

US EPA (US Environmental Protection Agency), 1994. 10-day chronic toxicity test using *Daphnia magna* or *Daphnia pulex*. EPA SOP#2028, Environmental Response Team, United States Environmental Protection Agency, Washington, D.C.

US EPA (US Environmental Protection Agency), 2002. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. EPA-821-R-02-012, United States Environmental Protection Agency, Washington, D.C.

Utermöhl, H, 1958. Zur Vervollkommnung der quantitativen Phytoplankton Methodik. Mitt. Int. Ver. Theor. Angew. Limnol. 9: 1-38.

Van Dolah, F. M., D. L. Roelke, R. Greene, 2001. Health and ecological impacts of harmful algal blooms: risk assessment needs. Hum. Ecol. Risk. Assess. 7: 1329-1345.

Wetzel, R.G. and G.E. Likens, 1991. Limnological analysis. Springer, New York, USA.

Wetzel, R.G., 2001. Limnology, 3rd ed. Academic Press, New York.

Wright, S., D. Thomas, H. Marchant, H. Higgins, M. Mackey, D. Mackey, 1996. Analysis of phytoplankton of the Australian sector of the Southern Ocean: comparisons of microscopy and size frequency data with interpretations of pigment HPLC data using the 'CHEMTAX' matrix factorization program. Mar. Ecol. Prog. Ser. 144: 285-298.

FIGURE CAPTIONS

FIGURE 1. Lake Whitney, Texas (USA). Experiments on *Prymnesium parvum* population dynamics were performed near Walling Bend Island, where high *P. parvum* population densities are historically observed, and a fish-killing bloom was developing during the study.

FIGURE 2. In-lake biotic parameters sampled during the six weeks of experiments in Lake Whitney. *P. parvum* population densities (dashed line) , and phytoplankton biomass (solid line), estimated as chlorophyll *a* concentration, with corresponding CHEMTAX percentages showing Prymnesiophyte dominance (A). Total in-lake zooplankton biovolume decreased with the developing bloom (B). Background shading relates to in-lake toxicity conditions: solid gray = non-toxic, white = moderately toxic, and dotted gray = highly toxic.

FIGURE 3. Ambient Lake Whitney toxicity to *Pimephales promelas* 48-h survival and *Daphnia magna* 10-d reproduction (neonates female $^{-1}$) at the initiation of three studies performed during spring 2007. NOAEL = no observed adverse effect level.

FIGURE 4. In-lake physicochemical variables monitored to determine effects on *P. parvum* population dynamics during experiments in Lake Whitney, February 21 to March 28, 2007. Dissolved inorganic nitrogen (NO_x = sum of nitrate and nitrite) and PO_4 concentrations suggest that nutrients where not limiting growth (A). Relatively deep readings and minor changes in Secchi depths recorded (B). Temperature steadily increased (solid line), and slight changes were observed in pH levels (dashed line, C).

FIGURE 5. Comparison of natural assemblage and reduced grazer treatments, showing initial conditions (filled squares) and data following 7-d experimental duration (bars) for experiments initiated February 22 (A), March 8 (B), and March 29 (C). Averaged *P. parvum* population densities (light gray bars); only experiment 3 demonstrated a significant difference between treatments ($p = 0.038$) for *P. parvum*. Averaged phytoplankton biomass, estimated as chlorophyll *a* concentration (dark gray bars). Average zooplankton biovolume and community composition (stacked bars), showing rotifer dominance in all three studies.

FIGURE 6. Comparison of rotifer zooplankton biovolumes in the natural assemblage and reduced grazer treatments for all three experiments (A, B, C). Initial conditions (filled squares) and averaged biovolumes from experimental termination (stacked bars) highlight changes in composition after 7 days. Rotifers dominated assemblages at termination of all experiments, shifting almost exclusively to *Notholca laurentiae* during the final experiment.

FIGURE 7. Comparison of natural assemblage and virus-reduced treatments for the final two experiments only (A, B), illustrating initial conditions (filled squares) and data after 7-d

experimental duration (bars). Averaged *P. parvum* population densities (light gray bars), where initial *P. parvum* conditions were adjusted, based on chlorophyll *a* concentrations, for the virus-reduced waters manipulated by tangential flow filtration. Only in experiment 3 was the difference between treatments significant ($p = 0.02$) for *P. parvum*. Averaged phytoplankton biomass, estimated as chlorophyll *a* concentration (dark gray bars). Average zooplankton biovolume and community composition (stacked bars), showing rotifer dominance in both experiments.

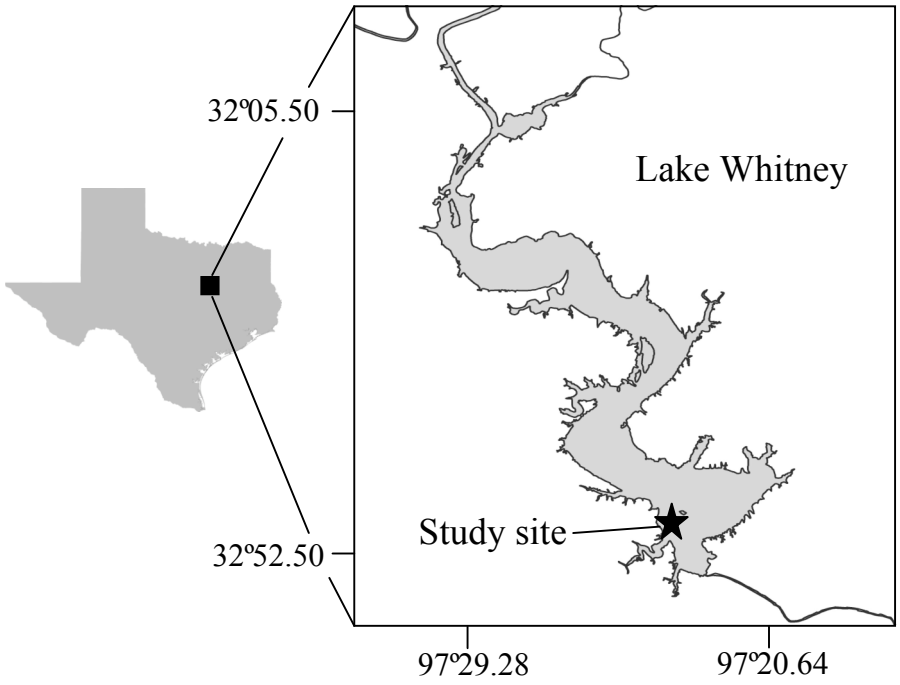

Fig. 1

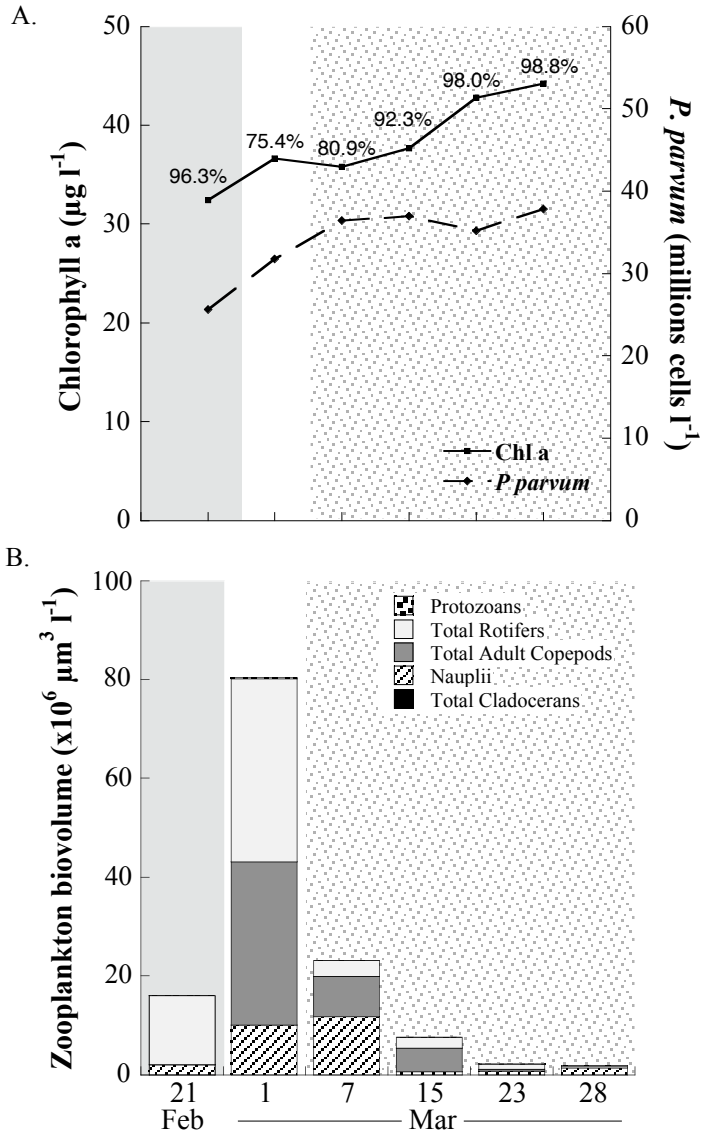
A.
Chlorophyll a (µg l-1)
P. parvum (millions cells l-1)
96.3%
75.4%
80.9%
92.3%
98.0%
98.8%
Chl a
P parvum
B.
Zooplankton biovolume (x10^6 µm^3 l-1)
Protozoans
Total Rotifers
Total Adult Copepods
Nauplii
Total Cladocerans
21
1
7
15
23
28
Feb
Mar

Fig. 2

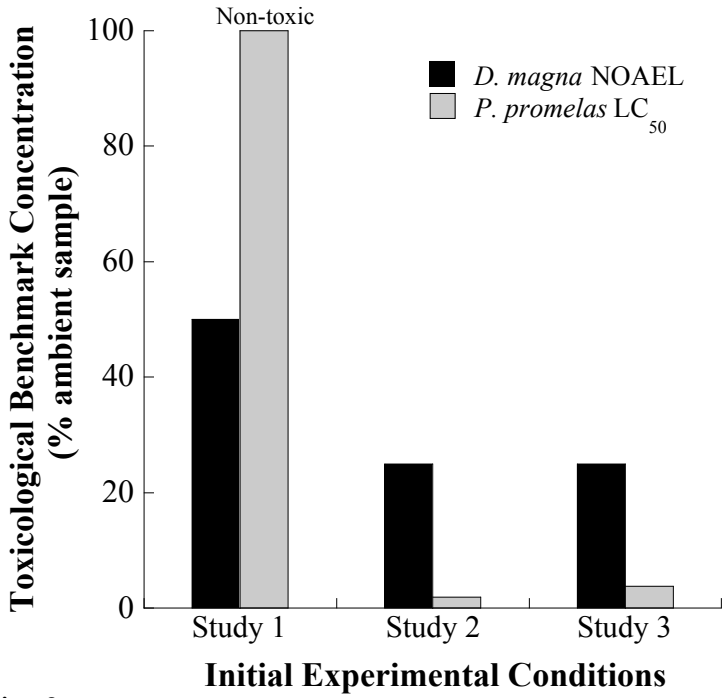
Non-toxic
100
80
60
40
20
0
Toxicological Benchmark Concentration
(% ambient sample)
D. magna NOAEL
P. promelas LC50
Study 1
Study 2
Study 3
Initial Experimental Conditions

Fig. 3

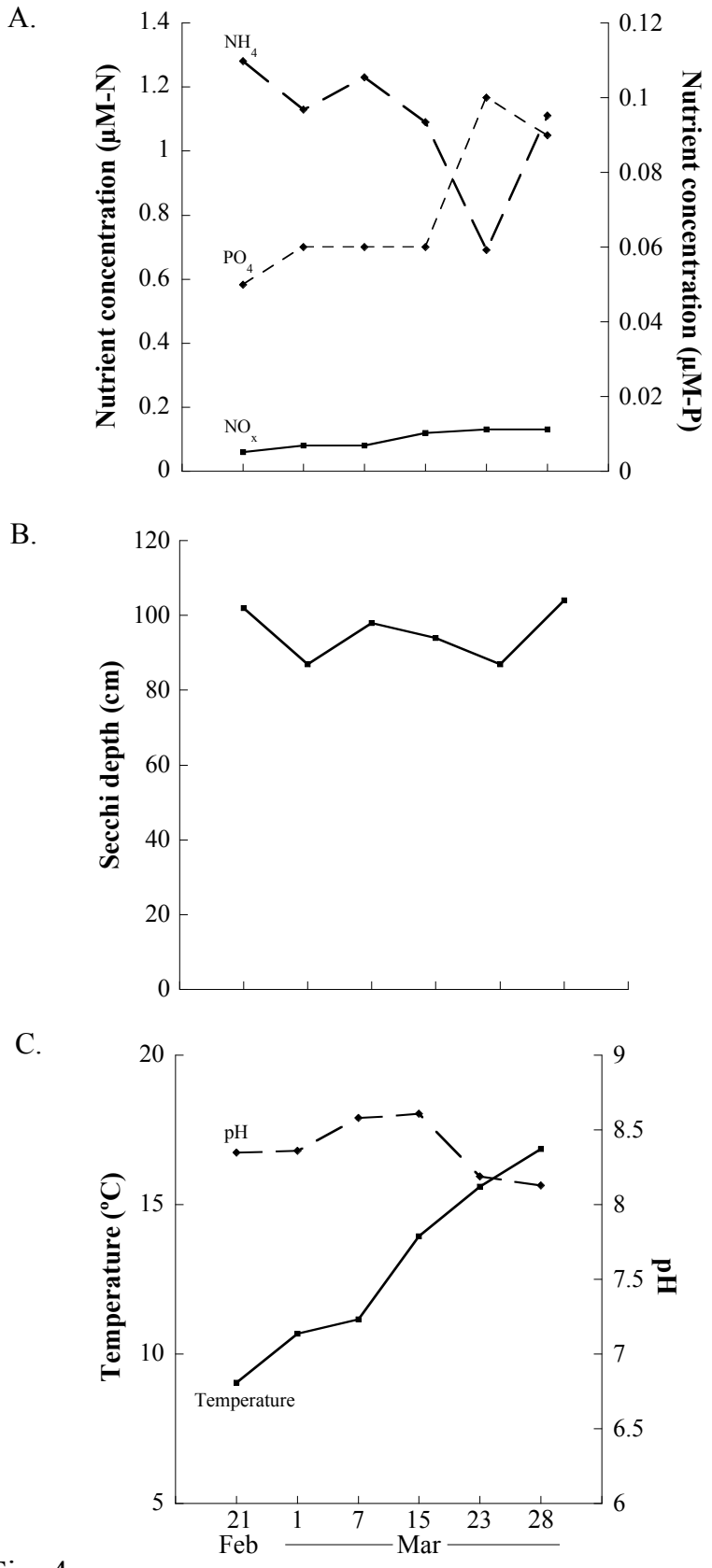
A.
Nutrient concentration (µM-N)
Nutrient concentration (µM-P)
NH4
PO4
NOx
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0.12
0.1
0.08
0.06
0.04
0.02
0
B.
Secchi depth (cm)
120
100
80
60
40
20
0
C.
Temperature (ºC)
pH
Temperature
20
15
10
5
9
8.5
8
7.5
7
6.5
6
21
1
7
15
23
28
Feb
Mar

Fig. 4

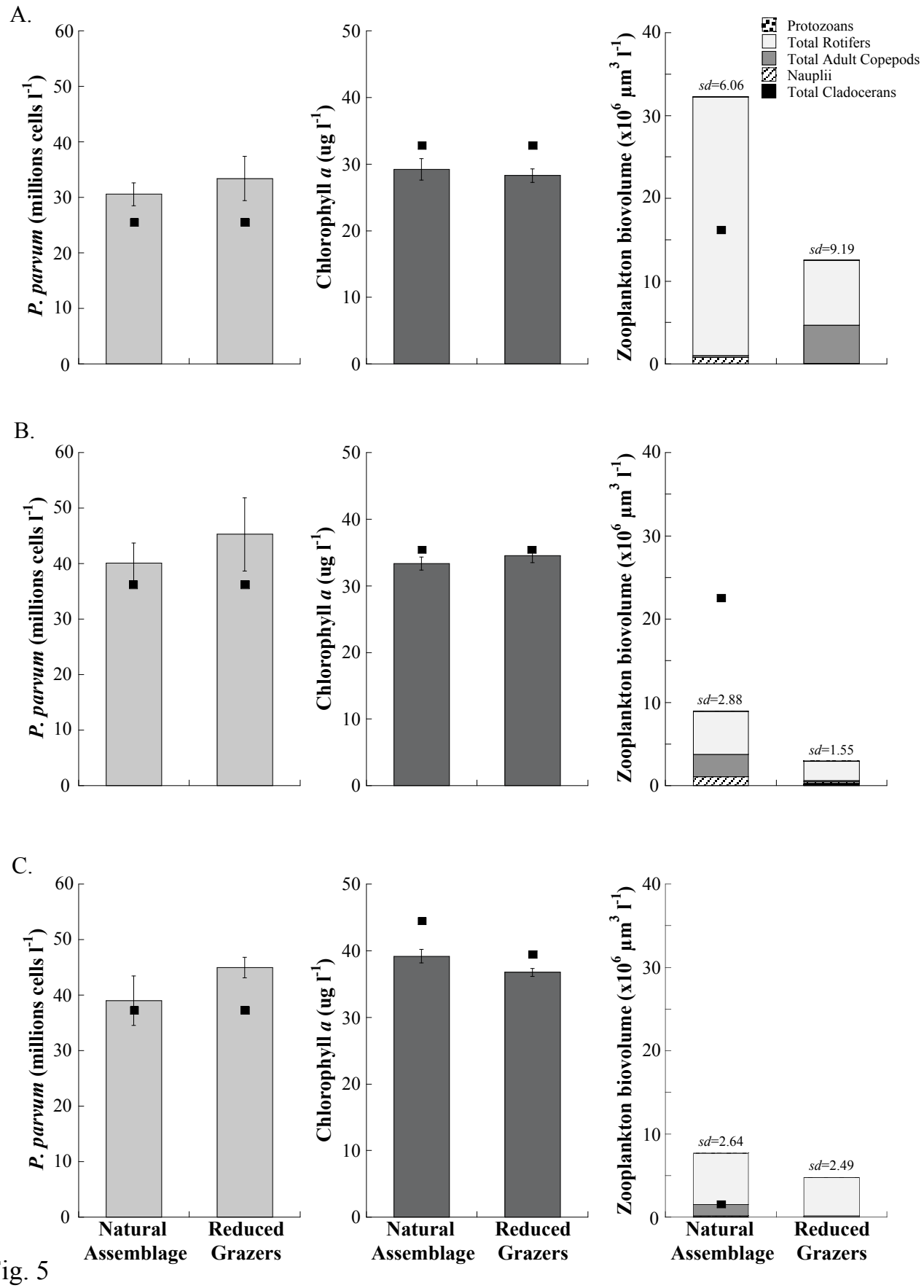
A.
B.
C.
P. parvum (millions cells l-1)
Chlorophyll a (ug l-1)
Zooplankton biovolume (x10^6 µm^3 l-1)
Protozoans
Total Rotifers
Total Adult Copepods
Nauplii
Total Cladocerans
sd=6.06
sd=9.19
sd=2.88
sd=1.55
sd=2.64
sd=2.49
Natural Assemblage
Reduced Grazers

Fig. 5

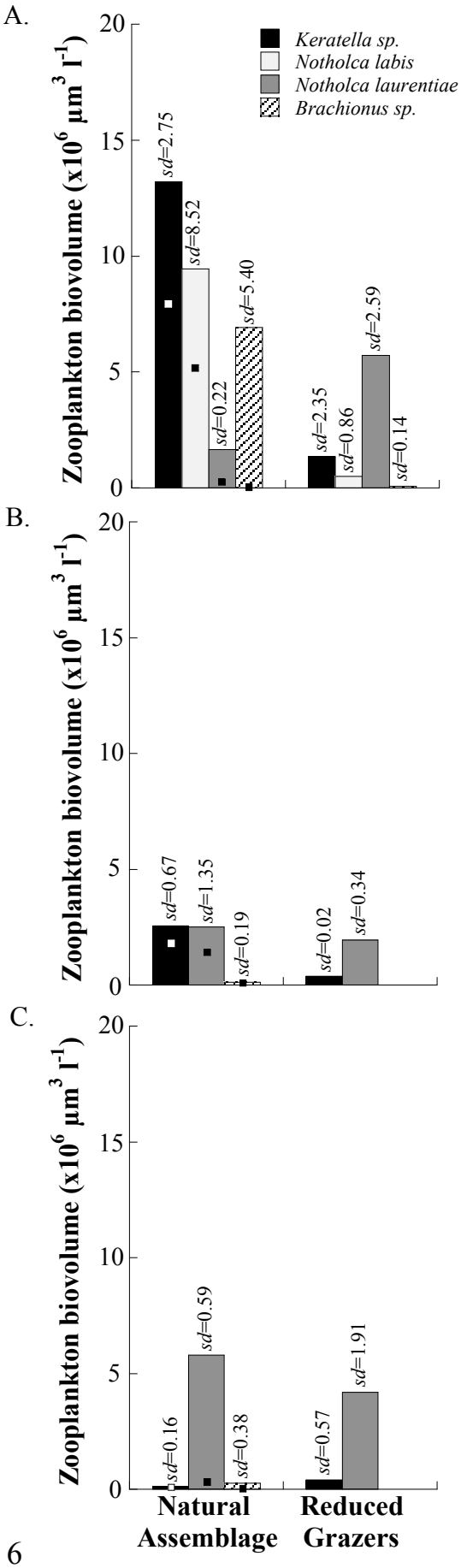
A.
B.
C.
Zooplankton biovolume (x10^6 µm^3 l^-1)
Keratella sp.
Notholca labis
Notholca laurentiae
Brachionus sp.
sd=2.75
sd=8.52
sd=0.22
sd=5.40
sd=2.35
sd=0.86
sd=2.59
sd=0.14
sd=0.67
sd=1.35
sd=0.19
sd=0.02
sd=0.34
sd=0.16
sd=0.59
sd=0.38
sd=0.57
sd=1.91
0
5
10
15
20
Natural Assemblage
Reduced Grazers

Fig. 6

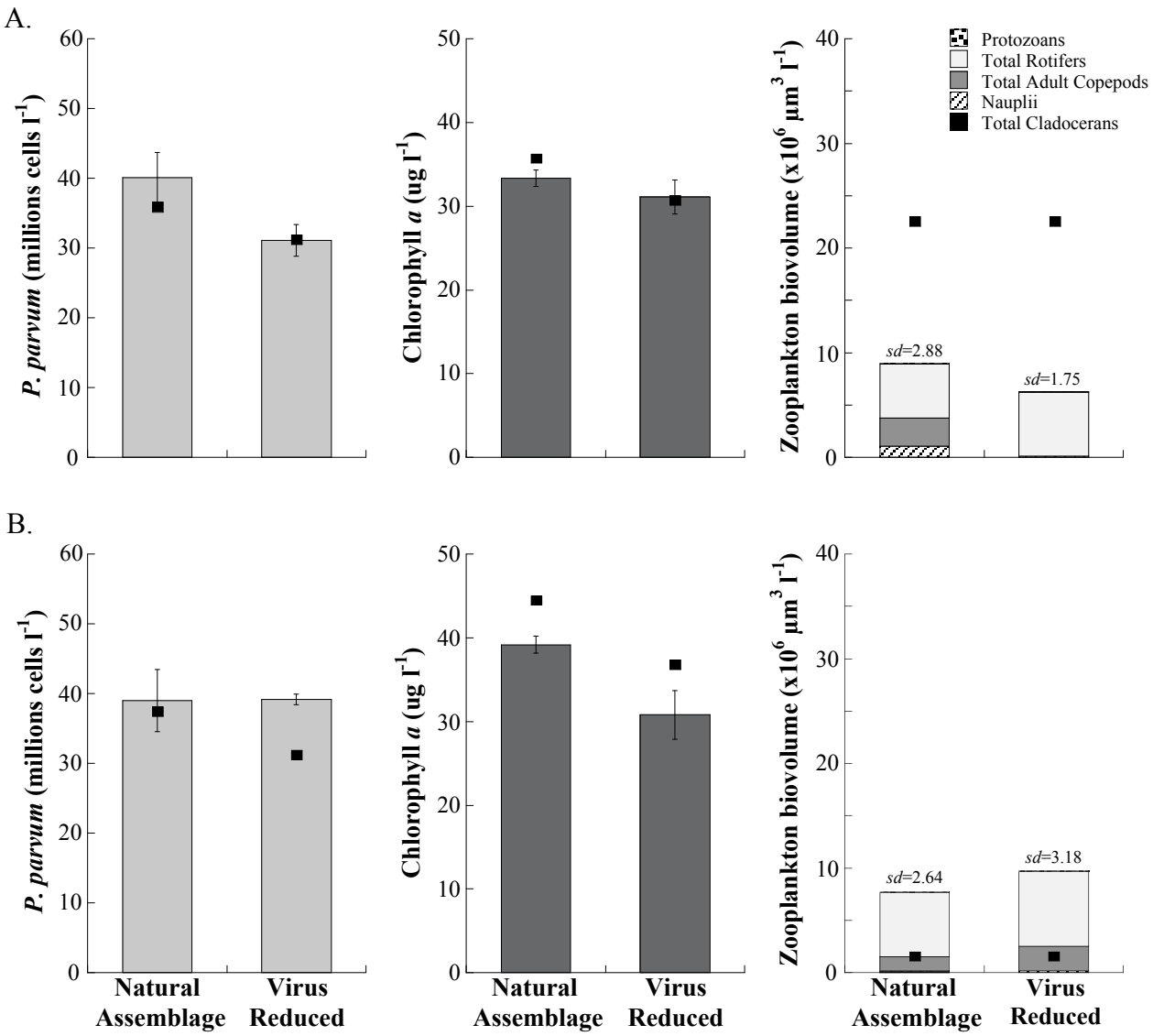
A.
B.
P. parvum (millions cells l-1)
Chlorophyll a (ug l-1)
Zooplankton biovolume (x10^6 µm^3 l-1)
Protozoans
Total Rotifers
Total Adult Copepods
Nauplii
Total Cladocerans
sd=2.88
sd=1.75
sd=2.64
sd=3.18
Natural Assemblage
Virus Reduced

Fig. 7